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AUTOMATABLE SCREENING OF YEAST ARTIFICIAL-CHROMOSOME LIBRARIES BASED ON THE %%%OLIGONUCLEOTIDE%%%-%%%LIGATION%%% ASSAY
KWOK P-Y; GREMAUD M F; %%%NICKERSON D A%%%; HOOD L; OLSON M V
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Automatable Screening of Yeast Artificial-Chromosome Libraries Based on the Oligonucleotide-Ligation Assay

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The systematic screening of yeast artificial-chromosome (YAC) libraries is the limiting step in many physical mapping projects. To improve the screening throughput for a human YAC library, we designed an automatable strategy to identify YAC clones containing a specific segment of DNA. Our approach combines amplification of the target sequence from pooled YAC DNA by the polymerase chain reaction (PCR) with detection of the sequence by an ELISA-based oligonucleotide-ligation assay (OLA). The PCR–OLA approach eliminates the use of radioactive isotopes and gel electrophoresis, two of the major obstacles to automated YAC screening. Furthermore, the use of the OLA to test for the presence of sequences internal to PCR primers provides an additional level of sensitivity and specificity in comparison to methods that rely solely on the PCR.

INTRODUCTION

The yeast artificial-chromosome (YAC) cloning system (Burke *et al.*, 1987), with its capacity to clone large (50- to >1000-kb) segments of DNA, is a powerful tool for the analysis of complex genomes. Large-scale screening of YAC libraries is most commonly performed by some combination of polymerase chain reaction (PCR) and colony-hybridization assays (Brownstein *et al.*, 1989; Green and Olson, 1990). PCR-based screening depends on assaying DNA prepared from pools of YAC clones for its ability to serve as the template in a PCR assay; the amplification products are analyzed by gel electrophoresis. Colony hybridization is often used to localize positive clones within small pools of YACs, once these pools have been shown to contain a positive clone by PCR analysis. Although this screening strategy is effective (Green *et al.*, 1991), it is labor intensive and requires, in its final steps, the use of radiolabeled probes. If colony hybridization is bypassed in favor of a purely PCR-based approach (Kwiatkowski *et al.*, 1990), gel electrophoresis becomes the rate-limiting step in YAC screening. To overcome this bottleneck, we have developed an automatable, nonradioisotopic method for screening large, ordered libraries of YAC clones.

The method is an extension of PCR-based screening that uses an ELISA-based oligonucleotide-ligation assay (OLA; Nickerson *et al.*, 1990) to detect the PCR products that contain the target sequence. Thus, both gel electrophoresis and colony hybridization are eliminated. The OLA employs two adjacent oligonucleotides: a "reporter" probe (labeled at the 5′ end with digoxigenin) and a 5′-phosphorylated/3′-biotinylated "anchor" probe. The two oligonucleotides, which are complementary to sequences internal to the PCR primers, are annealed to target DNA and, if there is perfect complementarity, the two probes are ligated together by T4 DNA ligase. Capture of the biotinylated anchor probe on immobilized streptavidin and analysis for the covalently linked reporter probe test for the presence or absence of the target sequences among the PCR products. The high sensitivity and specificity of this combined PCR–OLA approach have been demonstrated in two successful screens of a 69,000-clone YAC library (Brownstein *et al.*, 1989) for YACs containing particular sequence-tagged sites (STSs; Olson *et al.*, 1989).

The two STSs we used in this study were generated from λ clones prepared from DNA derived from a human–hamster hybrid cell line containing human chromosome 7. Both of the STSs, sWSS182 and sWSS198, have been described previously (Green *et al.*, 1991). They were chosen for the current study for two reasons. First, the PCR assays were of low specificity and yielded nonspecific products similar in size to the predicted product. Although their specificity could undoubtedly be improved by altering the PCR conditions, our experience with hundreds of YAC library screens indicates that there are too many false negatives when assays are tuned to high specificity. Since OLA detection of PCR products utilizes internal primers, one would expect OLA to offer an important new level of specificity to marginal PCR assays. Second, we wanted to explore further the behavior of sWSS182, which had been found to be pres-

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TABLE 1

Nucleotide Sequence of the STSs sWSS182, sWSS198, and the Corresponding PCR Primers and OLA Probes

sWSS182: 5'-AAGTTAGTGT GATTTGGGTG TTCNCCACTG GCATGTAAAA GAATTCTGAG
TAAAGCATAT GCCAATCATC AAGTATTTAT GAACCCAACT CTGTGACCAA
CATTAAAGAG GAAAAATAGA AATGACACTC ATGCCTGCTC TGGAGGAAAT
GACACTCTAT CTCCTTAACA GCAT-3'

sWSS198: 5'-GGGTATCAGT GTTATTGTTC CAATACCGAA GCAGATGTGG GCTCTTGCAT
CTCTGGTTTC TTATCATGTC CGTGGAGAAA CCAGTAATTC GTCTTCACTA
AAATTTTATT TATAGTTAGG CATGCGATCC ATGACCACAG AATAGAAT

STS	PCR primers	OLA probes[a]
sWSS182	5'-AAGTTAGTGTGATTTGGGTG	5'-D-ATTCTGAGTAAAGCATATGC
	5'-ATGCTGTTAAGGAGATAGAGTG	5'-pCAATCATCAAGTATTTATGA-B
sWSS198	5'-GGGTATCAGTGTTATTGTTC	5'-D-CATCTCTGGTTTCTTATCAT
	5'-ATTCTATTCTGTGGTCATGG	5'-pGTCCGTGGAGAAACCAGTAA-B

[a] D, digoxigenin; B, biotin, p, terminal phosphate.

ent in 16 1920-clone pools by PCR–gel electrophoresis. Fifteen of these pools were pursued in the original PCR/colony hybridization screening of the YAC library, and 11 of the pools yielded a total of 14 YAC clones (3 pools gave 2 YAC clones each). Since our YAC library on average should only give 6 YAC clones for each single copy sequence, we wanted to see if OLA detection could identify the false positive clones, if any, among these 14 YAC clones.

MATERIALS AND METHODS

YAC clones. Details about the construction and maintenance of the human YAC library, as well as the purification of DNA from pools of YAC clones, have been previously reported (Burke and Olson, 1991; Brownstein *et al.*, 1989; Green and Olson, 1990). Extraction of some of the YAC DNA was performed using a modification of a lithium dodecylsulfate-based procedure (Anand and Southern, 1990; MacMurray *et al.*, 1991; B. H. Brownstein, personal communication).

PCR assays. The *in vitro* amplification of specific target DNA sequences from pooled YAC DNA was performed as described by Green and Olson (1990). Primer sequences for the two PCR assays, sWSS182 and sWSS198, are shown in Table 1. The incubation mixture contained 2.5 m*M* $MgCl_2$, 50 m*M* KCl, 10 m*M* Tris (pH 8.3), 67 μM of each dNTP (dATP, dCTP, dGTP, dTTP), 0.42 μM of each oligonucleotide primer, 0.05 units of AmpliTaq polymerase (Perkin–Elmer/Cetus) per μl reaction, and 170 ng of bovine serum albumin (BRL) per μl reaction. Twenty-five microliters of light mineral oil was overlaid on the reaction mixture. Thermal cycling of incubation mixtures (30 μl, containing 100 ng of DNA) was performed using a DNA thermal cycler (Perkin–Elmer/Cetus) for 35 cycles using the cycling protocol of 92°C for 1 min, 60°C for 2 min, and 72°C for 2 min. An aliquot of the PCR products (10 μl) was removed for analysis by the OLA. To provide a comparison assay using standard procedures, 10 μl of the same PCR mixture were analyzed by polyacrylamide gel electrophoresis.

Synthesis of modified oligonucleotides. Instead of using the previously reported 3'-digoxigenin-labeled oligonucleotide, prepared by the occasionally unreliable terminal-transferase tailing reaction (Nickerson *et al.*, 1990), we took advantage of commercially available reagents to synthesize a 5'-digoxigenin-labeled oligonucleotide and a 3'-biotinylated oligonucleotide as OLA probes. Oligonucleotides used in the OLA were assembled on an Applied Biosystems Inc. (ABI) 394 DNA synthesizer. To prepare the 5'-digoxigenin-labeled oligonucleotide, an aminohexylphosphate linker (Aminolink 2, ABI) was added to the 5' end of the reporter probe during automated oligonucleotide synthesis as described by the manufacturer. After deprotection by treatment with ammonium hydroxide at 50°C overnight and purification by ethanol precipitation, the crude oligonucleotide bearing a primary amino group (100 nmol) was resuspended in 100 μl of 100 m*M* $NaHCO_3$/Na_2CO_3, pH 9. Digoxigenin-3-*O*-methylcarbonyl-ϵ-aminocaproic acid-*N*-hydroxy-succinimide ester (1.3 mg, 2 μmol, Boehringer–Mannheim, dissolved in 50 μl dimethylformamide, Clontech, HPLC grade) was added to the modified oligonucleotide and incubated overnight at room temperature (Zischler *et al.*, 1989). After evaporation to dryness under reduced pressure, the crude digoxigenin-labeled oligonucleotide was resuspended in 0.1 *M* triethylammonium acetate, pH 7, and directly applied to a reverse phase-C18 column for purification by high-performance liquid chromatography (HPLC). The 3'-biotinylated oligonucleotide was prepared by using a 3' biotin-ON CPG column (Clontech) as the starting "nucleotide" and was 5'-phosphorylated chemically with 5' phosphate-ON (Clontech) during automated oligonucleotide synthesis according to the manufacturer's directions. The biotinylated oligonucleotide can be used directly after purification by either ethanol precipitation or reverse phase HPLC.

OLA assays. A robotic workstation (Biomek 1000 workstation, Beckman) was used to assemble the ligation reaction mixtures and to perform the ELISA. The ligation reaction and the ELISA were performed as previously described (Nickerson *et al.*, 1990). Ten microliters of the PCR mixture was removed from under the mineral oil and placed in each well of a 96-well U-bottomed microtiter plate (Falcon). Forty-five microliters of 0.25 *M* NaOH containing 0.1% Triton X-100 was added to the amplified DNA samples. After 2 min, the mixture was neutralized with 45 μl of 0.25 *M* HCl containing 0.1% Triton X-100, bringing the final volume to 100 μl. Two 10-μl aliquots (each containing only 1 μl of the PCR mixture) were added to a 96-well U-bottomed flexible microtiter plate (Falcon) containing the OLA probes (200 fmol each) in 10 μl of 2× ligation buffer (100 m*M* Tris · HCl, pH 7.5/20 m*M* $MgCl_2$/2 m*M* spermidine/2 m*M* adenosine triphosphate/10 m*M* dithiothreitol) and 50% formamide. Samples were covered with 60 μl of light mineral oil, denatured at 93°C for 2 min, cooled to room temperature, and returned to the workstation for the addition of 5 μl of T4 DNA ligase (5 units/ml) (Boehringer–Mannheim) in 1× ligation buffer. The final ligation mixture volume was 25 μl. Ligations were performed at room temperature (RT) for 15 min. Reactions were stopped by adding 10 μl of 0.25 *M* NaOH/0.1% Triton X-100 per well and, after 2 min at RT, 4 μl of 3 *M* sodium acetate (pH 6.5) per well. Samples were transferred to a 96-well flat-bottomed microtiter plate (Falcon) coated with streptavidin [60 μl of Avidin-DX (100 μg/ml) (Vector Laboratories) for 1 h at 37°C] and blocked 20 min before use with 200 μl of 100 m*M* Tris · HCl, pH 7.5/150 m*M* NaCl/0.05% Tween 20 (buffer A) per well with 0.5% dry milk and 100 μg of

TABLE 2

Comparison of YAC Library Screening by PCR–Gel Electrophoresis/Colony Hybridization and PCR–OLA Using the STS sWSS198

1920-clone pool	PCR-gel electrophoresis	PCR-OLA	384-clone pool	PCR-gel electrophoresis	PCR-OLA	Colony hybridization	YAC clone	PCR-gel electrophoresis	PCR-OLA
4	++	++	4-A	++	++	++	A6C7	++	++
10	++	++	10-B	++	++	++	A88A10	++	++
12	−	+							
14	+	−							
15	++	++	15-D	++	++	++	B179B8	++	++
17	++	++	17-C	++	++	+	A114G9	++	++
			17-D	++	++	++	A119H3	++	++
			17-E	+	−	−			
18	++	++	18-E	++	++	++	B204H4	++	++
20	++	++	20-A	++	++	++	B225H10	++	++
23	++	++	23-D	++	++	++	A161A9	++	++
31	+	−							
35	++	++	35-C	++	++	++	D108H10	++	++
			35-E	++	++	++	D115E2	++	++

Note. Only the results for 1920-clone pools that were at least weakly positive by one or the other method are analyzed. For gel electrophoresis, − denotes no visible band, + a weak band, and ++ a strong band, at the predicted size, respectively. For PCR-OLA, − denotes an average absorbance of <0.2 units, + absorbances of 0.2 to 0.7 units, and ++ absorbances of >0.7 units, respectively. For colony hybridization, − denotes no signal above background, + a weak signal distinguishable from background, ++ strong signal with minimal background, respectively. Where a pool was not pursued by a particular method of detection, it was left blank.

salmon sperm DNA per ml (Sigma). Biotinylated OLA probes were captured at RT for 30 min before the wells were washed twice with 0.01 *M* NaOH/0.05% Tween 20 and once with buffer A to remove unbound OLA probes. Thirty microliters of anti-digoxigenin antibodies conjugated with alkaline phosphatase (Boehringer-Mannheim) diluted 1:1000 in buffer A containing 5% dry milk were added to each well. After incubation at RT for 30 min, unbound antibodies were removed by washing the wells four times with buffer A. Substrate (30 μl, BRL ELISA amplification system) was added, incubated for 15 min, followed by the addition of amplifier (30 μl). After 15 min of amplification, 0.3 *M* sulfuric acid (60 μl) was added to each well to stop the reaction, and spectrophotometric absorbances were taken at 488 nm by an automatic ELISA plate reader (Artek).

Southern analysis of YAC clones. Endonuclease digestion of 1 μg of DNA prepared from positive YAC clones was performed with *Eco*RI (10 units, Boehringer-Mannheim) in a 15-μl reaction for 2 h at 37°C. The DNA samples were separated on a 1.2% agarose gel in TBE by electrophoresis (500 Vh). The DNA was blotted onto Hybond-N membrane (Amersham) by alkali transfer. The membrane was probed with gel-purified sWSS182 PCR product radiolabeled with [^{32}P]dCTP by random hexamer labeling. Hybridization was performed overnight at 65°C. Following hybridization, the membrane was washed twice with 2× SSC at RT for 5 min and twice with 0.2× SSC containing 1% Sarkosyl at 65°C for 15 min. The membrane was then exposed to XAR film (Kodak) at −80°C for 3.5 days.

RESULTS

The general approach to screening the human YAC library with the PCR has been described elsewhere (Green and Olson, 1990). Briefly, individual clones are stored in 96-well microtiter plates. Clones from four such microtiter plates are grown on nylon filters (384 colonies per filter, 180 filters for the entire library). The yeast cells from each filter are pooled and the DNA is purified, yielding 384-clone pools of DNA. Equal aliquots of DNA from 384-clone pools are mixed together in groups of five to yield 36 1920-clone pools for the whole library. PCR assays are then used to analyze individually the 1920-clone pools for the presence of the target sequence. DNA from the human lymphoblastoid cell line CGM1, derived from the same individual whose DNA was used to prepare the YAC library, and DNA from a YAC clone containing an anonymous segment of yeast DNA as the cloned insert is included during the PCR analyses as positive and negative controls, respectively. If DNA from a 1920-clone pool is found to contain the target sequence, each constituent 384-clone pool is analyzed individually.

In the PCR-OLA modification of this procedure, we followed the same series of steps, substituting OLA for gel electrophoresis as the method for detecting amplified products. Furthermore, successively subdividing the YAC pools into 96-clone pools and 16-clone pools, we identified individual positive clones without recourse to colony hybridization. This pooling scheme replaces the colony hybridization step with three rounds of PCR (screening of 96-clone pools, 16-clone pools, and then pooled yeast cell PCR from the four rows and four columns that make up the 16-clone pools). Therefore, a total of five levels of PCR screening are needed to identify the well in which the YAC clone containing the STS resides. Yeast cells from the well are then streaked onto an AHC plate, and individual colonies are tested for the presence of the STS by colony PCR.

To demonstrate the feasibility of this approach, two anonymous STSs on human chromosome 7, sWSS182 and sWSS198, were used to screen a human YAC library by the PCR-OLA protocol (see Table 1 for the STS, PCR primer, and OLA probe sequences; detailed PCR conditions are found in Green *et al.*, 1991). The se-

TABLE 3

Comparison of YAC Library Screening by PCR–Gel Electrophoresis/Colony Hybridization and PCR–OLA Using the STS sWSS182

1920-clone pool	PCR-gel electrophoresis	PCR-OLA	384-clone pool	PCR-gel electrophoresis	PCR-OLA	Colony hybridization	YAC clone	PCR-gel electrophoresis	PCR-OLA
1	−	+			−				
2	+	++	2-E	++	++	++	B114E7	++	++
3	−	+	3-C		++				
4	++	++	4-E	++	++	++	A49A9	++	++
5	−	+			−				
9	++	++	9-D	++	++	++	A61C4	++	++
12	++	++	12-B	+	−				
			12-D	++	++	++	B105D7	++	++
13	++	++	13-A	++	++	++	B185A5	++	++
14	+	−		−					
16	++	++	16-B	++	++	++	B189A7	++	++
			16-E	++	++	+	B157H7	++	++
18	+	−	18-B	+		−			
21	+	++	21-B	+	++	+		−	−
23	++	++	23-B	++	++	−	A155E4		++
24	++	++	24-A	++	++	++	A171H5	++	++
25	+	++	25-C	++	++	−	A253D7		++
27	++	++	27-B	++	++	++	A133C4	++	++
							A133B8	++	++
28	++	++	28-B	++	++	−	B143A6		++
30	++	++	30-A	++	−	+		−	
			30-D	++	++	+	D11C8	++	++
			30-E	++	++	+		−	
31	++	++	31-A	++	++	+	D19E3	++	++
			31-B	++	++	++	D23F4	++	++
			31-E	+	++				
33	++	++	33-E	++	++	++	D78F7	++	++
34	+	−	34-A	+		−			

Note. Only the results for the positive 1920-clone pools are analyzed. See footnote of Table 2 for explanation of symbols used.

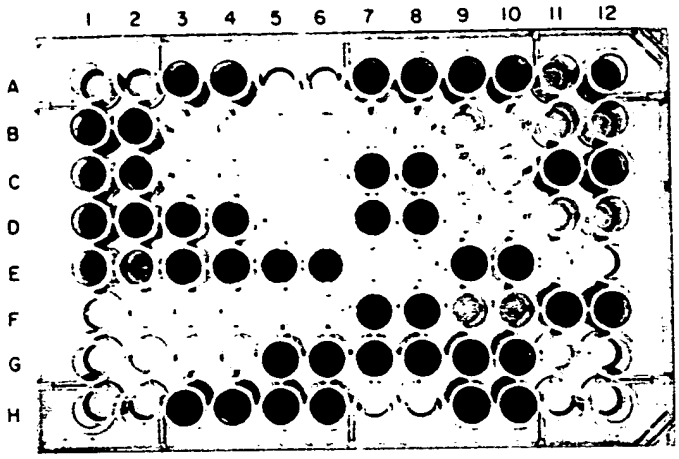
quences of the two STSs were obtained by one-pass, radioactive, dideoxy sequencing of one-sided PCR products subcloned into Bluescript. The OLA probes were chosen by inspection from a region of the sequence internal to the PCR primers that was without ambiguous base assignments. Care was also taken to make sure that the OLA probe sequences were not composed of extensive runs of one base or simple repeats. These STSs had been used previously to screen the same library by the method of Green and Olson (1990).

In the current study, DNA from all 36 1920-clone pools, as well as the DNA from selected 384-clone pools (those constituting the positive 1920-clone pools) and from individual YAC clones, was amplified by PCR and analyzed by OLA. In both cases, the PCR–OLA results agreed almost perfectly with the PCR/colony-hybridization results. A comparison of the results generated by PCR–gel electrophoresis and PCR–OLA is shown in Tables 2 and 3. Representative data from one of the two screens, i.e., that testing for the presence of the STS sWSS182, are presented in Fig. 1. Of the PCR assays performed, 48 are shown here tested in duplicate by OLA. The 48 assays included all 36 of the 1920-clone pools, 9 of the 384-clone pools that were subpools of three of the positive 1920-clone pools (23, 25, and 28) that did not yield positive YAC clones by colony hybridization, as well as positive (human DNA) and negative (yeast YAC DNA) controls. Twelve of the 48 gel lanes are presented for comparison. All YAC pools shown to contain the expected 174-bp PCR product by gel electrophoresis (pools 2, 4, 9, and 12) were also strongly positive in the OLA (wells B1/B2, D1/D2, A3/A4, and D3/D4). For YAC pools with only weakly positive OLA results (wells A1/A2, C1/C2, and E1/E2), faint 174-bp bands were detected by gel electrophoresis (pools 1, 3, and 5).

The three YAC pools giving weakly positive PCR–OLA signals (1920-clone pools 1, 3, and 5) were pursued by PCR–OLA but gave negative results at subsequent levels of screening (Table 4). The four YAC pools that gave positive signals at the level of the 384-clone pool but failed to give a positive signal upon colony hybridization (384-clone pools 21-B, 23-B, 25-C, and 28-B) were screened by PCR–OLA using pooled DNA (see Table 4). Three of these pools yielded YAC clones containing sWSS182, while one (pool 21-B) failed at the 96-clone pool level by PCR–OLA.

The presence of sWSS182 in these YAC clones was confirmed by Southern analysis of the *Eco*RI digest of all 17 YAC clones probed with sWSS182. All 17 YAC clones gave a single positive 1-kb fragment, while an anonymous YAC clone found on chromosome 14 similarly digested gave no positive fragments.

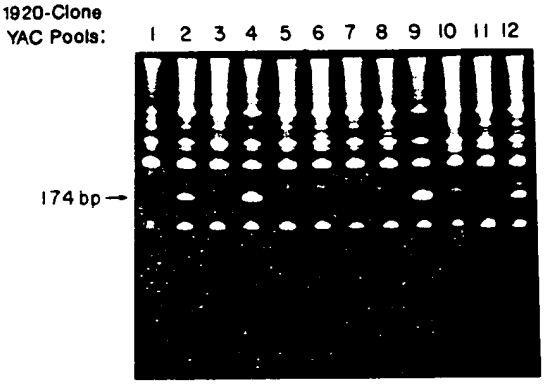
A



B

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.2	0.3	1.3	1.3	0.1	0.1	1.3	1.3	1.3	1.3	0.2	0.3
B	1.3	1.3	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.2
C	0.6	0.6	0.0	0.0	0.0	0.0	1.3	1.3	0.1	0.1	1.3	1.3
D	1.3	1.3	1.3	1.3	0.0	0.0	1.3	1.3	0.1	0.0	0.2	0.2
E	0.5	0.5	1.3	1.3	0.8	0.8	0.0	0.0	1.3	1.3	0.0	0.0
F	0.0	0.0	0.0	0.0	0.0	0.0	1.3	1.3	0.3	0.3	1.3	1.3
G	0.0	0.0	0.0	0.0	1.3	1.3	1.3	1.3	1.2	1.0	0.0	0.0
H	0.0	0.1	1.3	1.3	1.3	1.3	0.0	0.0	1.3	1.3	0.0	0.0

C



1920-Clone YAC Pools	OLA Wells
1	A1/A2
2	B1/B2
3	C1/C2
4	D1/D2
5	E1/E2
6	F1/F2
7	G1/G2
8	H1/H2
9	A3/A4
10	B3/B4
11	C3/C4
12	D3/D4

FIG. 1. (A) Identification of YAC pools containing the STS sWSS182 by OLA. PCR-amplified samples from YAC pools were analyzed in duplicate using the ligation probes for sWSS182 (see Table 1). Wells containing immobilized digoxigenin form a magenta-colored product that indicates complementarity between the ligation probes and amplified DNA. Wells A1/A2 to D9/D10 (from top to bottom by pair of columns) contain amplified DNA from the 1920-clone pools 1 to 36, analyzed in duplicate; wells E9/E10 to E11/E12, DNA from the 384-clone pools 23-B, C, D, 25-C, D, E, and 28-B, C, D, respectively; wells F11/F12 contain amplified DNA from human genomic DNA as a positive control, while wells G11/G12 and H11/H12 contain amplified DNA from a YAC clone with a yeast insert as a negative control. (B) Spectrophotometric absorbance readings of individual samples taken at 488 nm by an Artek V-Beam plate reader with well H12 taken as blank. The boxed region shows the readings for which corresponding electrophoretic assays are shown in C. (C) Electrophoresis of PCR products. Lanes **1–12** contain amplified DNA from 1920-clone pools 1–12, respectively. The expected product size for sWSS182 is 174 bp. Pools 2, 4, 9, and 12 gave strong signals, pools 3 and 5 gave faint signals, and pool 1 gave a very faint signal for the 174-bp product. The ELISA absorbances of wells containing the corresponding OLA products from these pools correlate well with the intensities of the 174-bp band detected by gel electrophoresis.

DISCUSSION

This study highlights the advantages of the PCR-OLA screening approach over the methods currently in use. These advantages are high sensitivity, high specificity, potential for automation, and the use of nonradioactive reagents. While 10-μl aliquots of a standard PCR mixture were used for electrophoretic assays of the amplified products, only 1 μl was needed in each OLA sample. Thus, while the functional sensitivity is comparable for PCR-OLA and PCR-gel electrophoresis in the present implementation, because only 10% of the amplified product was assayed, the potential for increased sensitivity is high.

Regardless of the screening method, there is an element of arbitrariness in choosing the threshold for a sig-

TABLE 4

PCR–OLA Screening of YAC Library Using the STS sWSS182 for Clones That Could Not Be Localized to a Single Well by PCR–Gel Electrophoresis/Colony Hybridization

1920-clone pool	384-clone pool	96-clone pool	16-clone pool	Row/column	YAC clone
1 +	–				
3 +	3-C ++	–			
5 +	–				
21 ++	21-B ++	–			
23 ++	23-B ++	A155 ++	A155-2 ++	E ++/4 ++	A155E4
25 ++	25-C ++	A253 ++	A253-3 ++	D ++/5 ++	–
				/6 ++	–
				/7 +	A253D7
				/8 +	–
28 ++	28-B ++	B143 ++	B143-1 ++	–/–	
			B143-3 ++	A ++/6 ++	B143A6

Note. See footnote of Table 2 for explanation of symbols used.

nal that is considered positive. We chose 0.8 absorbance unit as the threshold (which corresponded to a strong signal on a gel), but we pursued all YAC pools giving an OLA signal of 0.2 absorbance units over control. In this study, all 3 YAC pools giving OLA signals of <0.8 absorbance units over control became negative in subsequent steps, while only 1 out of 15 YAC pools (at the 1920-clone level, pool 21) giving OLA signals of 0.8 or more absorbance units did not yield a positive YAC clone. The problem of occasional pools that are erratically positive has been observed with all YAC-screening protocols. It is presumably due to low-level DNA contamination of pools and mixed clones in the master well with slight representation of the true-positive clone (the YAC library was made up of clones picked from crowded transformation plates without subsequent colony purification).

The high specificity of the OLA is illustrated by the results obtained by screening the YAC library for sWSS182. Even though the PCR assay had low specificity under the conditions used, only those pools yielding the expected 174-bp product were positive by OLA. A good example of the specificity of OLA is provided by the 1920-clone pool 10 (Fig. 1C, lane 10), where several faint bands around the expected 174-bp size were detected electrophoretically. However, close inspection indicated that the 174-bp band was absent in the sample and the corresponding OLA analysis (Fig. 1A, wells B3/B4) was unambiguously negative. The added level of specificity provided by the internal OLA probes eliminates the need for expert interpretation of gels and should enable the use of less stringent PCR conditions for screening, thereby mitigating the need for assay-by-assay optimization of PCR conditions.

For a single-copy STS to be found in 17 or more YAC clones in a library of approximately six- to sevenfold genomic redundancy is unexpected ($P = 0.00043$). We have not yet determined whether this result is due to nonrandomness in the library or the presence of more than one copy of sWSS182 on human chromosome 7. However, the concordance between the electrophoretic and OLA assays, as well as the demonstration that sWSS182-homologous sequences are present on a 1-kb *Eco*RI fragment in all clones, indicates that if sWSS182 does detect more than one site on chromosome 7, the boundaries of the duplication extend considerably beyond the STS itself.

As it is presently implemented, one technician can process 192 PCR samples by OLA in 3 h, which is comparable to the rate of processing samples by gel analysis. However, the skills required in performing the OLA and interpreting the results are less demanding than those required in gel analysis. The automated format would allow high-throughput screening of the YAC library with minimal handling of samples or reagents. Since all the reagents utilized in the PCR–OLA protocol are nonradioactive, the laboratory management of large-scale screening projects would also be simplified.

The potential drawbacks of the PCR–OLA approach include the need for sequence information for the selection of PCR and OLA primers and the added expense of preparing the two modified oligonucleotide probes for OLA. However, as the use of STSs becomes widespread in physical and genetic mapping, sequence information will be widely available. Furthermore, the expense of the OLA is easily offset by the savings in skilled technical labor.

In summary, the sequence-based PCR–OLA strategy provides an automatable, sensitive, and specific method for the screening of YAC libraries. Given the demands for large-scale YAC library screening that are posed by projects to map whole mammalian chromosomes, these are critical experimental attributes.

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